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Biomedical Engineering

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Transformation into NEB 5-alpha Competent E. Coli

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1 Transformation into NEB 5-alpha Competent E. Coli

Estimated bench time: 30 minutes

Estimated total time: 20 hours

Purpose: Placing the plasmid into bacteria for amplification after Gibson Assembly.

1.1 Materials

- Agar plates
- Bucket with ice
- Falcon tube
- Heat/shock machine
- Mini centrifuge
- NEB 5-alpha competent E. Coli bacterial cells
- Pipettes and tips
- Plasmid DNA
- SOC medium

1.2 Setup & Protocol

- Thaw a tube of NEB 5-alpha Competent E. coli cells on ice until the last ice crystals disappear. Mix gently and carefully pipette 50 µl of cells into a transformation tube on ice.
- Add 1-5 µl containing 100 pg-1 µg of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex.
- Place the mixture on ice for 30 minutes. Do not mix.
- Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.
- Place on ice for 5 minutes. Do not mix.
- Pipette 950 µl of room temperature SOC into the mixture.
- Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
- Warm agar plates to 37°C.
- Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
- Spread 50-100 µl of each dilution onto an agar plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 24-36 hours or 25°C for 48 hours.